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Human Papillomavirus Infection in Lung vs. Oral Squamous Cell Carcinomas: A Polymerase Chain Reaction Study

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Abstract: The role of Human Papillomavirus (HPV) has been suspected in pathogenesis of various malignancies; however, the available data are not conclusive. This study aimed to determine and compare the frequency of HPV infection in oral and lung Squamous Cell Carcinoma (SCC) by a sensitive method. Sixty specimens of oral and lung SCC (30 cases each one) were reevaluated in Tabriz Imam Reza Centre in a 24 month period. Following genomic DNA extract, the Polymerase Chain Reaction (PCR) amplification was performed in presence of specific MY11 and MY09 primers for HPV infection. Three cervical specimens and a combination of PCR solution lacking DNA plus healthy persons' DNA samples were employed as positive and negative controls, respectively. The oral group was significantly older than the lung group (68.90 vs. 56.67 y, $p < 0.001$) with more males in the latter (83.3 vs. 60%; $p = 0.04$). Percentages of HPV infection in the oral and lung groups were comparable (20 vs. 10%, respectively; $p = 0.47$). Majority of patients with HPV infection were older than 60 years (88.9%) or male (88.9%). In the oral group, all these cases were well differentiated and the majority was of lower lip origin (83.3%). In the lung group, 66.7% of these specimens were moderately differentiated and the origin was bronchus in all cases. In conclusion, the rate of HPV infection in lung and oral SCC samples is rather lower than the previous reports in the literature. This rate is apparently higher in the oral than the lung SCC specimens.

Key words: Papillomavirus infection, squamous cell carcinoma, lung, oral cavity, polymerase chain reaction

INTRODUCTION

Lung cancer is one the most common malignancies all over the world (Oskoei and Mahmoudian, 2007; Safdar and Khan, 2003; Abdullah *et al.*, 2009). Although, it is generally believed that smoking is the most potent risk factor of this malignancy, scientists believe that other possible environmental factors may contribute to its development (Mountain, 1997). Oral cancers constitute another group of frequent malignancies (Muralinaidu *et al.*, 2008). It is estimated that these cancers with pharyngeal malignancies altogether are the sixth most frequent cancers in the world leading to 2% of all deaths occur due to malignant conditions (Abraham *et al.*, 2009). Again, the pathophysiology and underlying etiologies are complex and not well-recognized. One of important culprits both in lung and oral Squamous Cell Carcinomas (SSCs) is human papillomavirus (HPV) (Greenlee *et al.*, 2000). HPV is a cutanotropic infectious agent associated with cervical dysplasias and malignant changes in the skin and other sites such as esophagus, nasal sinuses, bladder,

upper and lower respiratory tracts and oral mucosa (De-Vita *et al.*, 2008; Bohlmeier *et al.*, 1998; Abo El-Maged *et al.*, 2005). There are a number of studies evaluated the frequency of HPV infections in lung or oral SSCs. However, the data are heterogeneous and inconclusive (Delavarian *et al.*, 2010; Mancilla *et al.*, 2011). This might be due to different racial susceptibility to HPV infection and consequent malignancies (Gatoo *et al.*, 2011). To the best of our knowledge, there is not any report on both oral and lung SSCs simultaneously regarding to the rate of HPV infection. The Polymerase Chain Reaction (PCR) has been accepted as a sensitive method in diagnosis of HPV infection in different samples (Moosavi *et al.*, 2008). This study aimed to determine and compare frequencies of HPV infection in specimens of oral and lung SSCs by the PCR method.

MATERIALS AND METHODS

Subjects: In this analytic-descriptive study, 60 specimens of oral (30 cases) and lung (30 cases) SSCs were

evaluated from July 2009 to July 2011. The specimens were retrieved from the archive of Pathology Department, Imam Reza Teaching Hospital, Tabriz, Iran.

Specimens and PCR: Sixty 10% formalin-embedded specimens of oral and lung SCCs were selected. Five micrometer slides were prepared and stained by standard hematoxylin and eosin (H and E). After deparaffinizing, the Phenol-chloroform (PC) extraction method was employed for isolating HPV DNA. The extracted DNA was served as a fragment to be amplified by the PCR process. The employed primers were HPV-specific and included MY09 (5'-CGT CC(AC) A (AG) (AG) GGA (AT) AC TGA TC-3') and MY11 (5'-GC(AC) CAG GG (AT) CAT AA (CT) AAT GG-3') sequences. These primers cover a wide variety of HPVs including types 6, 11, 16, 18, 31, 33, 45, 51, 52, 56, etc.,. The PCR products were run on agarose gel and the results of electrophoresis were documented. Amplification of HPV-specific bands was considered as a positive consequence (HPV positive case) (Fig. 1). Three cervical specimens were employed and served as the positive controls. The PCR solution lacking genomic DNA and healthy persons' DNA samples were employed and served as negative controls.

Study design and variables: Frequency of HPV infection was compared between the two groups. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences. The studied data were patients' demographics, grade and location of tumor and HPV infection.

Statistical analysis: Statistical evaluation was made using SPSS for Windows V 18.0 (SPSS Inc., IL, USA). Data were

shown as frequency (percentage) or Mean±SD. Independent samples t, chi-square and Fishers' exact tests were employed for statistical analysis. The $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

The mean age of patients in the oral cancer group was significantly higher than that in the lung cancer group (68.90 ± 8.51 vs. 56.67 ± 11.68 , $p < 0.001$). There were significantly more males in the lung cancer group (83.3% vs. 60%; $p = 0.04$). The two groups were comparable with regard to the tumor grade (Table 1).

Although, the percentage of HPV positive cases was higher in the cases with oral SCC than in the patents with lung SCC, this difference was not statistically significant (6 cases (20%) vs. 3 cases (10%), respectively; $p = 0.47$) (Fig. 2). A sample of PCR results in detection of HPV infection is depicted in Fig. 1. In this peculiar case the results of PCR are assessed for HPV types 16, 31, 33, 45, 51, 52 and 56 (Fig. 1).

The majority of patients (8 out of 9; 88.9%) with HPV infection were older than 60 years or male (8 out of 9; 88.9%). In the oral cancer group, all the cases were well differentiated and the majority was of the lower lip origin (5 out of 6; 83.3%). Two out of 3 (66.7%) cases with the lung cancer were moderately differentiated and the origin of specimen was bronchus in all these 3 cases (Table 2).

In this study, frequency of HPV infection was determined and compared in specimens of cases with oral and lung SCCs. This rate was 10% in lung SCC group vs. 20% in oral SCC specimens with no significant difference ($p = 0.47$). Possible association between HPV infection and lung cancer was proposed when scientists reported HPV-induced like morphological lesions (condylomatosis) in bronchial mucosa of near 25% of

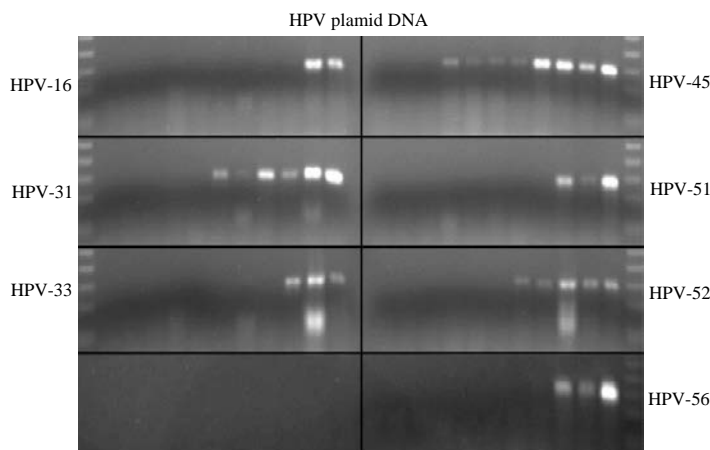


Fig. 1: Agarose gel electrophoresis of polymerase chain reaction products of various human papillomavirus types

Table 1: General data of patients with oral and lung squamous cell carcinomas

Variable	Oral cancer (n = 30)	Lung cancer (n = 30)	p-value
Age (year)	68.90±8.51 (48-85)	56.67±11.68 (35-92)	<0.001
Gender			
Male	18 (60)	25 (83.3)	0.04
Female	12 (40)	5 (16.7)	
Grade			
Poorly differentiated	2 (6.7)	2 (6.7)	0.77
Moderately differentiated	3 (10)	5 (16.7)	
Well differentiated	25 (83.3)	23 (76.7)	
Location			
Lower lip	13 (43.3)	Bronchus 21 (70)	-
Tongue	11 (36.7)	Lung 9 (30)	
Oral mucus	4 (13.3)		
Palate	2 (6.7)		

Data are shown as mean±standard deviation (range) or frequency (percentage). p<0.05 is considered statistically significant

Table 2: Patients' characteristics with *Human papillomavirus* infection

Patient No.	Group	Age	Gender	Grade	Location
1	Oral cancer	59	Male	Well differentiated	Lower lip
2	Oral cancer	65	Male	Well differentiated	Tongue
3	Oral cancer	74	Male	Well differentiated	Lower lip
4	Oral cancer	75	Male	Well differentiated	Lower lip
5	Oral cancer	75	Male	Well differentiated	Lower lip
6	Oral cancer	77	Female	Well differentiated	Lower lip
7	Lung cancer	67	Male	Well differentiated	Bronchus
8	Lung cancer	62	Male	Moderately differentiated	Bronchus
9	Lung cancer	66	Male	Moderately differentiated	Bronchus

patients with lung SCC (Chang *et al.*, 1992). Evidences of HPV infection in laryngeal and nasopharyngeal SCCs (Furuta *et al.*, 1992), as well as a higher susceptibility for HPV infection (Yousem *et al.*, 1992) in smokers further encouraged this hypothesis of association. These reports were the basic reasons for carrying out the present study in two common SCCs in human being in respect to the infection rate of HPV. The rate of HPV infection by PCR evaluation of specimens of lung SCC ranges between 5.9-30% in the United States (Al-Ghamdi *et al.*, 1995; Fong *et al.*, 1995), 11% in France (Thomas *et al.*, 1996a, b) 0-79% in Japan (Kinoshita *et al.*, 1995; Szabo *et al.*, 1994; Hirayasu *et al.*, 1996; Miyagi *et al.*, 2000; Kaya *et al.*, 2001), 9-80% in other south-east Asian countries (Bejui-Thivolet *et al.*, 1990; Liu *et al.*, 1994; Sagawa *et al.*, 1995; Xing *et al.*, 1993; Syrjanen, 2002; Nakazato *et al.*, 1997) and 26% in Iran (Nadji *et al.*, 2007a, b). Many different factors may justify this wide range of reports (0-80%). Comparing with these reported figures, percentage of HPV infection in the lung SCC specimens in the present study (10%) also lies in this range; however, a wide variation is clearly apparent. As seen, the geographic variation is a distinct feature in this regard; indicating possible racial and ethnical heterogeneity either for susceptibility for HPV infection or possible consequent malignancies. The reported rate in Iranian series is from northern regions with inhabitants (Mazandarani) genetically and racially different from the studied population in present study (Azeri). This might be

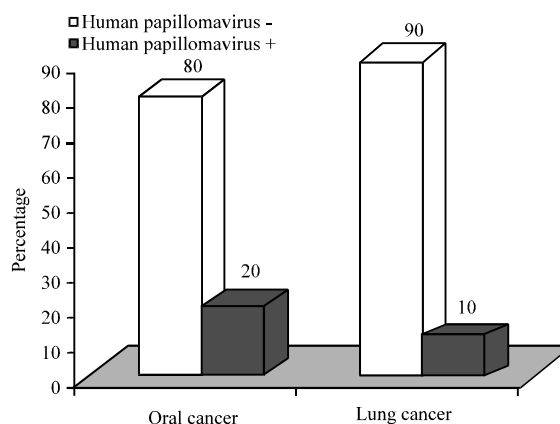


Fig. 2: Percentage of cases with human papillomavirus according to the polymerase chain reaction testing in oral and lung cancer groups

the cause of difference between the reported figures in our series and other studies from Iran. The rate of HPV infection in patients with oral SCC is 1.4% in Africa (Van Rensburg *et al.*, 1996), 29%-67% in the United States (Miller *et al.*, 1994; Summersgill *et al.*, 2000), 22-90% in Taiwan (Chen *et al.*, 2002; Luo *et al.*, 2007), 33.6% in India (Nagpal *et al.*, 2002), 75% in Brazil (Xavier *et al.*, 2005) and 74% in China (Zhang *et al.*, 2004). In two meta-analyses by Miller and Johnstone (2001) and Kreimer *et al.* (2005) the rates ranged between 12.9% and 55% in different areas. The rate of HPV infection in the oral SCC specimens in our series, in comparison, also falls in this range. Apparently, there is a wide range of reports in this regard, too. Previously mentioned justifications about the geographical and ethnical variations may be evident here. Chewing tobacco has been known to cause cancer, particularly of the mouth and throat. This behavior also increases susceptibility for HPV infection. There is known geographic variation for this habit (Parkin *et al.*, 1999). So, the current results in our series could be best judged after performing further studies in the same region. In the

current study the rate of HPV infection was compared between the patients with oral and lung SCCs. To the best of our knowledge, there is not any similar report in the literature. According to our findings, the rate of HPV infection was twice more common in the oral cancer cases; however there was no statistically significant difference. This may be due to closer location of oral cavity to the body surface than the lungs. In other words, it may be hypothesized that probability of infection of oral cavity is more than that in deeper located lower respiratory system. Further studies will elucidate this hypothesis.

CONCLUSION

Based on results of the current study, 20% of patients with oral SCC and 10% of patients with lung cancer have HPV infections in their cancerous squamous cell epitheliums. Comparing with previous reports, the rate of infection is lower in our population. Longitudinal cohort studies on healthy people with HPV infection may be helpful to further elucidating of association of this organism and oral/lung carcinomas.

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